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Pathway in Breast Cancer

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14. ABSTRACT This proposal seeks to generate SABs (synthetic antigen binders) against the PRLr (prolactin receptor) signaling complex to systematically inhibit and modulate its important activities in breast cancer. This complex has clearly been demonstrated to play a significant role in the development and spread of this disease, and yet the generation of pharmacologic agents that can specifically block their function has been slow. We hypothesize that SABs can be rapidly generated to the PRLr, CypA, CypB, Jak2, Stat3, and Stat5 and delivered into the cell, where these reagents will block breast cancer cell growth, survival, and spread. We have successfully generated phage to Cyp A and B, and have begun to characterize SAB against the PRLr. It is the intent of this proposal to validate the efficacy of these highly innovative reagents on a panel of breast cancer cells in vitro, with future translation into pre-clinical testing.						
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Introduction and Scope of Research: Our research objective is to develop a novel set of technologies that will target the Jak/Stat signaling cascade in breast cancer. These technologies, which involve a new class of affinity reagents and intramolecular delivery tools, have the capability of identifying the most important nodes in this signaling pathway and ultimately inhibiting or modifying them to influence effects on breast cancer cell proliferation and death. The interplay between the Jak and Stat components in cytokine signaling has been an area of intense investigation. However, although many of the molecular interactions that occur between them and with other signaling partners have been broadly implicated in breast cancer, they are poorly characterized because of a lack of appropriate experimental tools. Consequently, a host of basic questions remain to be answered. Our goal is to develop an experimental framework to sort out the most important interactions in the pathway and establish whether there is a specific Achilles Heel that can be exploited to attack breast cancers in innovative ways. As a long-term goal, we will utilize this information to develop novel synthetic antibody reagents that can be delivered with precision and potency to breast cancer cells.

Research Accomplishments: Aim 1- Generating synthetic antigen binders (sABs) to components of the prolactin receptor signaling network. Ultimately, we will want to generate reagents that directly bind and antagonize Jak2- prolactin receptor binding as a means to down regulate receptor signaling. However, there are other protein components in the system besides Jak2 that we postulate could also be interfered with to effectively abrogate receptor signaling and which are readily available for evaluation. In this first six month period we picked four components to focus on using sAB technology to: 1) block binding of prolactin (hPRL) to the extracellular domain (ECD) of its cognate receptor (hPRLR), 2) inhibiting CypA, 3) inhibiting CypB, and blocking Jak2/Stat5. CypA and CypB are proline isomerase enzymes that play critical roles in signaling; in the case of CypA to switch on the kinase activity of Jak2 and for CypB to assist in activation Stat5 in the nucleus.

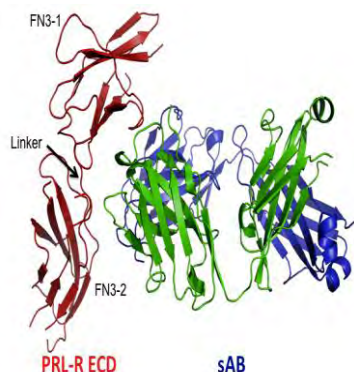


Figure 1- X-ray crystal structure of the hPRLR extracellular domain (ECD) bound to an inhibitory sAB. The receptor ECD has two fibronectin domains connected by a short linker. The sAB binds across the two domains. Interestingly, the known hormone binding site is actually on opposite face of the ECD. Thus, antagonism is generated through indirect effects, not direct blocking of the hormone binding site. The mechanism of hormone binding inhibition is based on the sAB altering the juxtaposition of the two fibronectin domains in a way that changes the hormone binding site. We note, that other inhibitory sABs might work through other mechanisms, like directly blocking the hormone binding site.

a) We have cloned, expressed and purified milligram quantities of CypA and CypB. We will employ phage display mutagenesis to generate sABs that inhibit the activity of these enzymes. We have developed a sorting protocol that selects sABs that block the active sites of these enzymes. We are currently in the second round (out of three) of selection and have generated a number of candidate sABs that meet the required criteria. After round three, we will pick 10 candidate sABs for each target (i.e. CypA and CypB) and evaluate their binding using surface plasmon resonance (SPR). sABs that bind to the enzymes with Kds lower than 10 nM will be used in the biological assays described in Aim 2.

b) We have completed phage display selection for sABs that bind to the ECD of hPRLR at sites that interfere with hormone binding. We theorized that this class of sABs will inhibit receptor signaling in cell-based assays by antagonizing hormone binding. Four candidate sABs that met our criteria were evaluated for binding affinity using SPR. All the sABs had Kds less than 30 nM, suggesting that they had potential application as potent hPRLR antagonists. To understand the mechanism through which the sABs might block hormone-receptor binding, we chose one of the sABs to determine a high resolution X-ray crystal structure analysis of the hormone-receptor complex. The structure is shown below.

c) We will use these inhibitory sABs in cell-based assays to evaluate their effectiveness as receptor antagonists.

Key Research Accomplishments

- Selected phage directed against CypA and B.
- Generated and characterized sAB against the PRLr-ECD.

Reportable Outcomes

In the first 6 months of funding of this proposal no reportable outcomes have been generated.

Conclusion

The reagents that are being generated are of exceptional importance to both basic and translational science in that they will provide a means of delivering inhibitory probes intracellularly into viable cells. The generation of sABs as outlined above has been successful and is following the timeline identified in the SOW.

References – None.

Appendices – None.

Supporting Data – None.